mechanisms of tissue injury may exist. Antibodies against minor histocompatibility antigens develop in association with cGVHD, suggesting a role for B cells. We therefore began a clinical trial of specific anti-B-cell therapy with rituxan for steroid-refractory cGVHD. **Methods:** Subjects had steroid-refractory (> 0.5 mg/kg prednisone each day for 4 weeks), clinically extensive cGVHD on stable doses of immunosuppressants. Rituxan (375 mg/m^2/week × 4) was followed by a second course for nonresponders or partial responders 4 weeks later. All patients underwent thorough oral, ocular, rheumatologic, and dermatologic examinations by subspecialists before and 8 weeks after rituxan therapy. A validated symptom scale was administered before and after rituxan therapy. **Results:** 16 patients (median age, 42.5 years) were treated, 15 of whom were on steroids (4 alone, 7 with 1 immunosuppresant, and 4 with 2 immunosuppressants). One patient was on tacrolimus alone. End-organ involvement included skin (in 13 patients), eye (in 8 patients), musculoskeletal (in 8 patients), and oral mucosa (in 5 patients). Six patients received 1 and 10 patients received 2 4-week courses of rituxan at a mean of 13.7 months (range, 2.9–82 months) from cGVHD diagnosis and 24.6 months from transplantation (range, 8.5–114.6 months). There were 5 grade 3 adverse events (3 cases of gastroenteritis, 1 case of hepatitis B reactivation, and 1 case of conjunctivitis) and 1 infusion reaction. Eight weeks after starting rituxan, CD19+ B cells were undetectable in all peripheral blood samples, median serum IgG levels fell by 19.8%, and IgM levels fell by 33%. Median follow-up was 160 days. Objective responses were noted in 10 patients (62.5%, all skin/musculoskeletal responses), and one patient had progressive cutaneous cGVHD. There was 1 cGVHD flare after a partial response and 1 malignant relapse. The median reduction in clinical cutaneous involvement was 52%, and the median reduction in the rheumatologic VAS pain score was 87.5%. Mean symptom scores improved in 7 patients (6 of whom had clinical responses), were unchanged in 7 patients, and worsened in 2 patients. As a result of cGVHD responses, prednisone doses were decreased in 11 patients and unchanged in 4 patients (median dose reduction, 50%). At this time, all patients remain on systemic immunosuppression. **Conclusions:** Rituxan is safe in patients with cGVHD and is active against cutaneous and musculoskeletal cGVHD. B-cell immunity contributes to at least some of the clinical manifestations of cGVHD.

30

HYPERACUTE GVHD: ANALYSIS OF RISK FACTORS, CLINICAL MANIFESTATIONS, AND OUTCOMES

Couriel, D.R.; Saliba, R.; De Lima, M.J.; Hosing, C.; Andersson, B.; De Jesus, J.; Mickler, K.; Hsu, Y.; Shpall, E.J.; Neumann, J.; Ghosh, S.; Champlin, R. University of Texas M.D. Anderson Cancer Center, Houston, TX.

We evaluated the clinical characteristics and risk factors for hyperacute graft-versus-host disease (GVHD) in 815 consecutive patients transplanted at M.D. Anderson Cancer Center between January 1998 and September 2002. Our definition of hyperacute GVHD included all patients with a diagnosis of GVHD within 14 days after allogeneic bone marrow or peripheral blood stem cell transplantation (PSCT). Of 381 patients presenting with clinical evidence of acute GVHD, 22% (n = 83) occurred within 14 days posttransplantation (early GVHD), and were all biopsy-proven. Grade I GVHD occurred in 12% of these patients, grade II in 53%, grade III in 13%, and grade IV in 22%. The proportion of grade II-IV GVHD in this group was significantly higher (88%) than in the 298 patients presenting with GVHD between days 15 and 100 posttransplantation (64%) ($P < .001$). The majority of patients with early GVHD had skin involvement (89%), followed by gastrointestinal (43%), and liver GVHD (19%). Skin involvement was significantly more common (89% vs 76%; $P = .01$), and more severe (stage III or IV 63% vs 32%; $P < .001$) in the early GVHD group. Significantly factors on univariate analysis included a graft from a mismatched (MM) related donor (HR = 4.4; $P < .001$) or a matched unrelated donor (MUD) (HR = 2.4; $P < .001$) vs a graft from a matched related donor, and myeloablative preparative regimen with or without total body irradiation (HR = 2.5; $P < .001$). These effects remained significant when evaluated in a multivariate model. Donor's age > 40 years was associated with a marginally significant lower rate of early GVHD (HR = 0.6; $P = .05$), and there was a trend toward increased rates for patients receiving sex-mismatched grafts, patients transplanted for solid tumors, and those having received more than 5 chemotherapy regimens before transplantation. There was no difference in the distribution of CD34+ or CD3+ cells infused. All-cause mortality rate was significantly higher within 6 months posttransplantation for patients with early GVHD (HR = 2.2; $P < .001$) compared with all other patients or to patients developing acute GVHD after day 14 (HR = 1.6; $P = .009$). Acute GVHD death rate was also significantly higher (HR = 2.3; $P = .007$). Hyperacute GVHD occurs in a substantial proportion of patients undergoing HSC transplant, even before neutrophil engraftment. Skin involvement, grades 3–4 GVHD, and higher mortality are common features of this syndrome.

31

ASSESSING DONOR CHIMERISM LEVEL AMONG CD3 T, CD4 T, CD8 T, AND NK CELLS PREDICTS SUBSEQUENT GRAFT REJECTION, GVHD, AND RELAPSE AFTER ALLOGENEIC HCT WITH NONMYELOABLATIVE CONDITIONING

Baron, F.[1]; Storb, R.[1]; Gooley, T.[1]; Sandmaier, B.[1]; Gisburne, S.[1]; Shin, S.[1]; Stroup, P.[1]; Baker, J.[1]; Maris, M.[1]; Maloney, D.[1]; Heimfeld, S.[1]; Grumet, F.C.[2]; Chauncey, T.[1]; Blume, K.[2]; Little, M.T.[1] 1. Fred Hutchinson Cancer Research Center, Seattle, WA; 2. Stanford University, Stanford, CA.

We previously showed that low levels of day-14 CD3 T and NK (CD56) cells donor chimerism predicted graft rejection, whereas high levels of day-28 CD3 T-cell donor chimerism predicted acute graft-versus-host disease (GVHD) after HCT with nonmyeloablative conditioning. Here we investigate whether assessing chimerism levels among CD4 T cells and CD8 T cells, and also the absolute number of lymphocyte subsets of donor and host origins, would lend greater precision to our initial observations. We analyzed data from 157 patients receiving HCT after conditioning with 2 Gy TBI +/− fludarabine as treatment for AML (n = 22), ALL (n = 4), CML (n = 13), CLL (n = 19), MDS (n = 26), MM (n = 24), NHL (n = 30), HD (n = 14), RCC (n = 4), and WASP deficiency (n = 1). Postgrafting immunosuppression included MMF and CSP. A total of 97 patients received grafts from HLA-identical siblings, and 60 patients received grafts from HLA-matched unrelated donors. Lymphocyte subsets were isolated from peripheral blood by flow cytometry on days 14, 28, and 42. The proportion of cells of donor origin (chimerism levels) were assessed by VNTR-PCR and quantified by phosphor imaging. Eighteen patients (11%) had graft rejection. Day-14 donor chimerism levels < 50% among CD3 T ($P = .0007$), CD4 T ($P = .03$), and NK cells ($P = .003$) but not CD8 T cells predicted graft rejection. High absolute numbers of CD3 T ($P = .002$) and NK cells ($P = .002$) of host origin on day 14 were each associated with increased risks of graft rejection when treated as continuous linear variables. Grades 2, 3, and 4 acute GVHD were seen in 40%, 9%, and 5% of patients, respectively. High donor chimerism levels on day 14 among CD3 T ($P = .02$), CD4 T ($P = .03$), and CD8 T cells ($P = .02$) but not NK cells were each associated with increased risks of grades 2–4 acute GVHD. High absolute numbers of CD4 T ($P = .04$) and CD8 T cells ($P = .04$) of donor origin on days 14–42 were each associated with increased risks of grade 2–4 acute GVHD when treated as continuous linear variables, whereas high donor CD3 T ($P = .002$), CD8 T ($P = .006$), and NK cell ($P = .002$) chimerism levels from days 14–42 were associated with decreased risks of relapse. No statistically significant correlations between absolute numbers of donor cells and risks of relapse were found. These data suggest that assessing CD3, CD4, CD8, and NK cell donor chimerism levels and determining absolute numbers of CD3 and NK cells of host and donor origins are useful for predicting HCT outcomes after nonmyeloablative conditioning.